sensory neurons from dorsal root (DRG) and trigeminal (TG) ganglia M channel activity is increased by the mitochondrial release of reactive oxygen species (ROS) induced by neuromodulator substance P. Here we identified a new signalling role for nitric oxide (NO) in TG sensory neurons. We show that in rat TG neurons, the NO donor, S-Nitroso-N-acetyl-DL-penicillamine (SNAP), inhibited M-current by $53 \pm 12\%$ (n=12). This inhibitory effect was blocked by scavenging of NO and inhibition of NO synthases increased M-current, suggesting that tonic NO levels inhibit M-current in TG neurons. NO increased release of calcitonin gene-related peptide (CGRP) from TG neurons ($130 \pm 10\%$ of control, $p \leq 0.05$), consistent with an increase in neuronal excitability. Importantly, incubation with the M-channel opener retigabine completely abolished increases in CGRP release. We further investigated the mechanism of the effects of NO on M channels and identified a site of action of NO to be the same triplet of cysteines, which is also a site of oxidative modification of M channels by ROS. We now show that the same triplet of cysteines can be S-nitrosylated in the presence of SNAP. We show that NO and oxidative modifications have opposing effects on M-current, suggesting that a tightly controlled local redox and NO environment will exert control over M-channel activity and thus neuronal excitability. Together our data have identified a dynamic redox sensor within neuronal M-channels which mediates reciprocal regulation of channel activity by S-nitrosylation and oxidative modification. This sensor may play an important role in controlling neuronal excitability by redox and NO-related mechanisms.

1381-Pos Board B273

Binding of ATP is Required for Opening of I_{Ks} Channels

Yang Li[1], Kelli Delaloye[1], Jingyi Shi[1], Kevin Bock[2], Kevin Tian[1], **Jianmin Cui**[1].
[1]Washington University in St. Louis, St. Louis, MO, USA, [2]Purdue University, West Lafayette, IN, USA.

The human heart demands a constant energy supply to fulfill its function, hence the decrease in myocardial ATP production plays a key role in the pathogenesis and progression of ischemic heart diseases. Ischemia also alters electrophysiology of the heart, exemplified by an association between an adverse prognosis and QT interval prolongation in acute myocardial ischemia. KCNQ1 and KCNE1 form the I_{Ks} potassium channel important in terminating cardiac action potentials. Congenital mutations that compromise I_{Ks} function prolong the duration of the ventricular action potential, causing Long QT (LQT) syndrome, which is associated with a high risk of sudden death. Here we report that the I_{Ks} channel activity increases with ATP concentration ([ATP]) and the EC_{50} is close to the physiological [ATP] in cardiac myocytes, which indicates that [ATP] changes such as in ischemia affect I_{Ks} channel function. Consistent with this observation, an LQT-associated mutation in KCNQ1 alters I_{Ks} function by reducing ATP sensitivity. The effect of this mutation is eliminated by increasing [ATP]. We find that GTP and a non-hydrolyzable ATP analog AMP-PNP can substitute for ATP in activating the channel, and an ATP analog can be photo-cross-linked to KCNQ1 proteins expressed in the membrane of *Xenopus* oocytes, indicating that the nucleotide directly binds to KCNQ1 to modify channel function. Compared to ATP, ADP and AMP are less effective in activating the I_{Ks} channel, suggesting that phosphate groups are important in nucleotide binding. Correspondingly, a mutational scan of all cytosolic basic residues shows that the ATP binding site may reside in the cytosolic C-terminal. These results demonstrate that I_{Ks} is a bona fide ATP activated potassium channel that modulates cardiac electrophysiology by sensing intracellular [ATP], thus connecting the cellular energy state to membrane excitability.

1382-Pos Board B274

Global Ischemia Upregulates KCNQ1 Potassium Channel Activity in Neurons

Kelly A. Aromolaran, Thomas V. McDonald, R. Suzanne Zukin.
Albert Einstein College of Medicine, Bronx, NY, USA.

Transient forebrain or global ischemia induces delayed cell death of hippocampal CA1 pyramidal neurons and impaired cognition. Potassium (K^+) channels are implicated as key players in ischemia-induced death. The KCNQ1 K^+ channel assembles with the auxiliary subunit KCNE1 to produce the slow component of I_k in the heart. Emerging evidence indicates that KCNQ1 mRNA and protein are expressed not only in heart, but also brain, although its function in neurons is, as yet, unclear.

The transcription factor REST (repressor element-1 silencing transcription factor)/NRSF (neuron-specific silencing factor) is upregulated in response to global ischemia and contributes to neuronal death. We recently showed that REST is enriched at the promoter of the KCNQ1 K^+ channel in postischemic CA1, assessed by targeted ChIP-chip. Here we set out to assess a possible role for KCNQ1 in ischemia-induced neuronal death. Global ischemia *in vivo* elicited an increase in KCNQ1 mRNA at 24 (~1.4 fold) and 48 h (~3.5-fold; $p < 0.04$), as assessed by qPCR. Moreover, ischemia induced an increase in KCNQ1 protein at 24 (~1.4 fold) and 48 h (~1.6 fold; $p < 0.004$), as assessed by Western analysis. To examine KCNQ1 function in brain, we identified and recorded K^+ currents from cultured hippocampal neurons with the specific KCNQ1 inhibitor Chromanol 293B (IC_{50}=62μM) and confirmed them with a dominant-negative KCNQ1 mutant S277L which reduced the endogenous current by ~50% ($p < 0.005$). Cultures exposed to oxygen-glucose deprivation (an *in vitro* model of ischemia) showed a marked increase in KCNQ1 currents at 48 h post ischemia (~28%, $p < 0.03$). These results document the presence of functional KCNQ1 channels in hippocampal neurons, and their upregulation in response to ischemia, consistent with a possible contribution to delayed neuronal death of the CA1.

1383-Pos Board B275

Protein Kinase C-Dependent Modulation of Heterologously Expressed Homomeric Kv7.4, Kv7.5 and Heteromeric Kv7.4/7.5; Differential Sensitivities to Low Concentrations of Vasopressin and PMA in A7R5 Vascular Smooth Muscle Cells

Lyubov I. Brueggemann, Kenneth L. Byron.
Loyola University Chicago, Maywood, IL, USA.

Kv7.4 and Kv7.5 voltage-activated potassium channels are proposed to contribute to the maintenance of resting membrane voltage in smooth muscle cells. We had previously provided evidence that Kv7.4 and Kv7.5 form predominantly heteromeric channels when natively or exogenously expressed in vascular smooth muscle cells. Endogenous Kv7 currents in smooth muscle cells are suppressed upon activation of G_q coupled receptors. It remained to be elucidated if both Kv7.4 and Kv7.5 respond similarly to low concentrations of vasopressin (AVP), an agonist of the $V_{1a}G_q$-coupled receptor. using patch-clamp techniques, we measured currents through human Kv7.4 and Kv7.5 channels expressed individually or together in A7r5 rat aortic smooth muscle cells and compared their sensitivity to AVP (100 pM and 500 pM) and to the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA, 1nM). AVP (100 pM) and PMA suppressed currents through Kv7.4, Kv7.5 and Kv7.5/7.5. Currents were reduced with different voltage dependencies and potencies in the rank order: Kv7.5 > Kv7.4/7.5 > Kv7.4. Both AVP and PMA increased the steepness of Kv7.5 voltage-dependent activation and dramatically decreased G_{max}. In contrast, AVP and PMA induced a rightward shift of the Kv7.4 activation curve with only a slight reduction in maximal conductance (G_{max}). The modulation of Kv7.4/7.5 activation by AVP and PMA had intermediate biophysical characteristics that were distinct from the modulation of either of the homomeric configurations. These findings reinforce the significance of PKC-dependent regulation of the Kv7 channels and suggest a differential regulation of Kv7.4 and Kv7.5 channel subunits by PKC-dependent phosphorylation.

1384-Pos Board B276

Kcnq Channels in Airway Smooth Muscle

Alexey I. Evseev, Iurii Semenov, Robert Brenner, Mark S. Shapiro.
University of Texas Health Science Center San Antonio, San Antonio, TX, USA.

The role of KCNQ (Kv7) channels is well established in neurons, where they play dominant roles in control of resting membrane potentials and cell excitability. Recent studies have revealed expression of KCNQ channels in different types of smooth muscle. Here we focused on airway smooth muscle (ASM), whose membrane potential is primarily, determined by BK-type K^+ channels. RT-PCR and immunostaining suggest that KCNQ 1, 4 and 5 are the predominant subtypes in rodent airway. To investigate the contribution of KCNQ channels in cholinergic-induced ASM contraction, we patch-clamped freshly isolated ASM cells and isolated KCNQ current as the non-inactivating component at the end of 2s depolarizations, with BK channels blocked with 1μM paxilline. The KCNQ current was enhanced by the KCNQ channel opener, flupirtine (10μM), and abolished by XE991 (10μM). Although XE991 depolarized the resting membrane potential, it did not affect that of ASM cells pretreated with carbachol. Similarly, XE991 did not effect carbachol-evoked contractions, whereas flupirtine induced a significant relaxation. The flupirtine effect is likely via opposing voltage-dependent Ca^{2+} influx, since no effect of flupertine occurred in a low K^+ (1mM) bathing solution. Pre-treatment with the muscarinic M_3-receptor antagonist, fumarate (5nM), to prevent $G_{q/11}$-mediated PIP_2 hydrolysis did not reveal an effect of XE991 on contractions, suggesting the mechanism of KCNQ inhibition in ASM is not due to PIP_2 depletion. Interestingly, an XE991-dependent increase in contractility was revealed in mice with the β1 BK channel subunit knocked out, an effect enhanced by the M_3 receptor antagonist. Thus, KCNQ channels may normally play a secondary role to BK channels in control of cholinergic evoked contractions in ASM; however, given that alteration in BK β1-subunit expression is known to occur in asthma, KCNQ channels in ASM cells may be potential targets for therapeutic intervention in respiratory disease.